REMARKS

The present Preliminary Amendment is submitted in order to correct some self-evident typographical errors and to eliminate multiple dependencies.

It is respectfully submitted that the claims are in condition for examination, and prompt and favorable action are earnestly solicited.

Respectfully submitted,

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"Version with markings to show changes"

IN THE SPECIFICATION

Page 7, please amend the first paragraph as follows:

The present invention provides an expression vector which, upon ~~using in~~ **insertion into** various host cells (particularly animal cells such as mammalian cells and insect cells), can secrete a recombinant protein produced extracellularly, allows the simple purification of the produced recombinant protein, and still further provides the recombinant protein almost identical in quality to the natural protein. The expression vector provided herein may also be used in situations where **it is preferred** to use microorganisms and the like as the host ~~is preferred~~, for example, where the presence of sugar chains on the protein is not necessary, or protein production is carried out as a basic study.

Page 15, please amend the first paragraph as follows:

After translation, an active protein may be obtained. Even when the resultant protein is not an active protein, it may be converted to an active protein by ~~applying~~ a variety of ~~processing~~ **techniques**. In many cases, a protein is first synthesized at the ribosomes in the cytoplasm as an inactive precursor (pro-form) which comprises an active protein bearing at the N-terminus thereof a peptide of about

15 to 60 amino acids responsible for secretion (secretory signal). The peptide region, which functions as a secretory signal, is concerned with the mechanism of passing through the cell membrane, and is removed by cleavage with a specific protease during the passage through the membrane (not always) to yield a mature protein. The peptide moiety which functions as a secretory signal has a broad hydrophobic region comprising hydrophobic amino acids in the middle of the sequence, and basic amino acid residues at a site close to the N-terminus. A secretory signal may be understood as a synonym of a signal peptide.

Page 15, please amend the second paragraph as follows:

In addition, in some proteins, a peptide moiety which functions as a secretory signal is further attached to the N-terminus of an inactive precursor (pro-form), and such a protein is called as a prepro-protein (the prepro-form). For example, trypsin is present as a prepro-form immediately after translation into amino acids, as a pro-form after being secreted from cells, and is converted into active trypsin in **the** duodenum upon limited degradation by enteropeptidase or by self degradation. A pro-form from which an active protein region has been deleted is called ~~as~~ a pro-region, a prepro-form from which a pro-form region has been deleted is called

~~as~~ a pre-region, and a prepro-form from which an active protein region has been deleted is called ~~as~~ a prepro-region.

Page 16, please amend the first paragraph as follows:

The "secretory signal nucleotide sequence", which is one of the essential components of the protein expression vector of the present invention, refers to the nucleotide sequence encoding a secretory signal. Also, the "secretory signal" refers to the pro-region when a protein **is** expressed as a pro-form, and at least the pre-region or the prepro-region when a protein **is** expressed as a prepro-form. However, the secretory signal is not limited in so far as it is capable of secreting the intracellularly expressed protein, extracellularly. The secretory signal nucleotide sequence constructed within the protein expression vector of the present invention preferably encodes a secretory signal with a cleavage site at the C-terminus of the signal. When the sequence encodes a secretory signal that does not contain a cleavage site at the C-terminus, it is preferred to newly insert a nucleotide sequence encoding a cleavable site at the 3' end of said secretory signal nucleotide sequence. This is, for example, a trypsin signal represented by 1st to 23rd amino acids in SEQ ID NO: 19. At the C-terminus (19th to 23rd amino acids) of said sequence, there is Asp-Asp-Asp-Asp-Lys which is

recognizable by enterokinase.

Page 17, please amend the first paragraph as follows:

Since the secretory signals of eukaryotic cells are similar to those of prokaryotic cells, *Escherichia coli* and the like may be used as the host. Since the secretory signal has different extracellular secretory activities depending on the host, it is necessary to select a secretory signal appropriate to the host. Specific examples of secretory signals include IgG (κ) (or IgGk) signal (or leader) and trypsin signal, which exhibit particularly high secretory activities when insect cells or mammalian cells are used as the host cells. Other examples of secretory signals include BiP of flies (*Drosophila*), melitin of honeybees, α-factor of Pichia pastoris, PHO, and the like. When a trypsin signal is referred to herein, it may be constructed by either the 1st to 18th amino acids or the 1st to 23rd amino acids in SEQ ID NO: 19. Further, the secretory signal also includes, other than those exemplified above, their homologs and variants which are capable of secreting proteins extracellularly.

Page 17, please amend the second paragraph as follows:

The "Tag nucleotide sequence", which is another essential component of the protein expression vector of the present invention, refers to a nucleotide sequence that encodes a Tag sequence. The "Tag sequence" refers to an amino acid sequence that is ~~no~~ not derived from the nucleic acid encoding a target protein and is inserted in order to facilitate, when expressed, isolation, purification and recognition of the target protein. Therefore, such a Tag sequence may be, for example, an antigen or an epitope recognizable by an antibody. By retaining the recombinant fusion protein containing a Tag sequence in a substance capable of recognizing said Tag sequence, isolation and purification can be carried out easily.

Page 24, please amend the first paragraph as follows:

Introduction of the above expression vectors into the host cells per se may be conducted by employing ~~one of~~ a conventional ~~methods~~ method which ~~include~~ includes, for example, transfection by the lipopolyamine method, the DEAE-dextran method, Hanahan's method, the lipofectin method, ~~and~~ the calcium phosphate method, microinjection, electroporation, and the like.

Page 26, please amend the second paragraph as follows:

Plasmid pSecTag2A (1 μg, 0.1 μl) was treated with the restriction enzymes Nhe I and BamH I to completely remove the region encoding IgGk leader sequence. To this solution were added 100 pmoles each of the sense DNA and the antisense DNA described above, and the mixture was treated at 70°C for 10 minutes, after which it was left standing at room temperature for 30 minutes to allow annealing. To 1 μl each of the His secretory signal sequence, which had been treated with Nhe I and BamH I, and pSecTag2A was added 2.0 μl of solution I of DNA Ligation Kit Ver. 2 (Takara Shuzo Co., Ltd.), and the mixture was allowed to react at 16°C for 30 minutes. To the reaction mixture was added 0.1 ml of competent *Escherichia coli* cells XL1-Blue (Stratagene Company), and the mixture was allowed to react on ice for 30 minutes, followed by heat shock at 42°C for 60 seconds. After the reaction mixture was left on ice for 2 minutes, 0.9 ml of the SOC medium (Toyobo Co., Ltd.) was added and the cells were shake-cultured at 37°C for one hour. The culture was centrifuged at 5,000 rpm for one minute and the supernatant was discarded. The sedimented competent cells ~~was~~ **were** suspended in the solution remaining in the centrifugation tube, and applied to two ampicillin LB plates containing 100 μg/ml ampicillin at a ratio of 1 : 10. The

cells were cultivated overnight at 37°C and, from plasmids obtained from the resulting colonies, those with inserted DNA of the His secretory signal were selected by PCR and designated as pTrypHis.

IN THE CLAIMS

3. (Amended) The protein expression vector according to claim 1 ~~or 2~~, wherein the cloning site or the nucleotide sequence encoding the target protein is present successively at the 3′ end of the cleavable nucleotide sequence.

4. (Amended) The protein expression vector according to ~~any one of claims 1 to 3~~ claim 1, wherein a nucleotide sequence encoding at least ~~on~~ one amino acid is contained as a spacer nucleotide sequence in the 3′ downstream side of the secretory signal nucleotide sequence, but in the 5′ upstream side of the cleavable nucleotide sequence.

6. (Amended) The protein expression vector according to claim 4 ~~or 5~~, wherein the spacer nucleotide sequence is composed of at least a cleavable nucleotide sequence.

7. (Amended) The protein expression vector

according to ~~any one of claims 1 to 6~~ claim 1, wherein the cleavable nucleotide sequence, when translated into an amino acid sequence, is cleaved by an enzyme at immediate upstream and/or immediate downstream and/or in the middle of said amino acid sequence.

9. (Amended) The protein expression vector according to claim 7 ~~or 8~~, wherein the enzyme is enterokinase.

10. (Amended) The protein expression vector according to ~~any one of claims 1 to 9~~ claim 1, wherein the secretory signal nucleotide sequence is an IgG (κ) signal or a trypsin signal.

11. (Amended) The protein expression vector according to ~~any one of claims 1 to 10~~ claim 1, wherein the Tag nucleotide sequence is polyhistidine.

12. (Amended) The protein expression vector according to ~~any one of claims 1 to 11~~ claim 1, further comprising a nucleotide sequence encoding an antibody recognition epitope.

13. (Amended) The protein expression vector according to ~~any one of claims 1 to 12~~ claim 1, wherein the nucleotide sequence encoding the target protein is that

encoding neurosin.

14. (Amended) Host cells transformed with the protein expression vector according to ~~any one of claims 1 to 13~~ claim 1.

18. (Amended) A process for producing a target protein which comprises using the protein expression vector ~~or the host cells~~ according to ~~any one of claims 1 to 18~~ claim 1.

20. (Amended) A process for producing a recombinant fusion protein comprising an amino acid sequence of a target protein which comprises using the protein expression vector or the host cells according to ~~any one of claims 1 to 18~~ claim 1.

22. (Amended) A process for producing a target protein which comprises retaining the recombinant fusion protein according to claim 21 with a substance capable of recognizing at least one of Tag and~~/or~~ an epitope in said recombinant fusion protein, liberating the recombinant fusion protein from the substance to purify it, and releasing the target protein by reacting said purified recombinant fusion protein with an enzyme capable of recognizing the cleavable site within said recombinant fusion protein, followed by collecting the released target protein.

23. (Amended) A process for producing a target protein, which comprises retaining the recombinant fusion protein according to claim 21 with a substance capable of recognizing **at least one of** Tag and~~/or~~ an epitope in said recombinant fusion protein, and releasing the target protein by reacting said purified recombinant fusion protein with an enzyme capable of recognizing the cleavable site within said recombinant fusion protein, followed by collecting the released target protein.

24. (Amended) A target protein is obtained by the process according to claim 22~~ or 23~~.